(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number
WO 02/00164 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/IB01/01133

(22) International Filing Date: 26 June 2001 (26.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
591/Mum/2000 26 June 2000 (26.06.2000) IN

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(81) Designated States *(national)*: AE, AG, AL, AU, BA, BB, BG, BR, BZ, CA, CN, CO, CR, CU, CZ, DM, DZ, EC, EE, GD, GE, HR, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MN, MX, NO, NZ, PL, RO, SG, SK, TT, UA, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
of inventorship (Rule 4.17(iv)) for US only

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CHEMOSENSITIZER

(57) Abstract: Chemotherapeutic agents are used to treat infections caused by bacteria, virus, protozoa, parasites, and various malignant diseases like cancer. The major problem associated with use of chemotherapeutic agents is resistance to chemoptherapeutic agents. Mechanisms underlying resistance to chemotherapeutic agents include inactivation/modification of antibiotic (beta-lactams, chloramphenicol), insensitive target site (beta-lactams, glycopeptides, macrolides, tetracyclines), decreased drug accumulation in the form of enhanced efflux (tetracyclines, chloroquine, macrolides, anticancer drugs), by-pass of antibiotic sensitive step (methicillin, sulphonamides) etc. The common mechanisms underlying drug resistance is to restrict concentration of drug at the site of action usually intracellular. This can be in the form or restricting the entry of the drug into the cell by various mechanisms including altered cell wall permeability. It can also be in the form of removing the drug from site of action e.g. intracellular so that therapeutic concentration are not achieved. The drugs restoring sensitiviy of chemotherapeutic agents are broadly known as chemosensitizers. Compounds belonging to a group of R-1-3-benzodiaxole are found to be chemosensitizer as per the present invention. Piperine is one such compound belonging to a group of R-1-3-benzodiaxole are found to be chemosensitizer as per the present invention. Piperine is one such compound belonging to R-1-3 benzodiaxole group. It is found to reverse resistance to chemotherapeutic agents like rifampicin at dose which is easiyl achievable after oral ingestion of the drug.

WO 02/00164 A2

THE PATENTS ACT, 1970
THE COMPLETE SPECIFICATION

1. CHEMOSENSITIZER.

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3. The following specification particularly describes the nature of this invention and the manner in which it is to be performed.

FIELD OF INVENTION

The objective of the present invention is to provide a chemosensitizer for therapeutic use.

The further objective of the present invention is to provide a chemosensitizer, the dose of which, as chemosensitizer is achievable.

BACKGROUND OF THE INVENTION

Chemotherapeutic agents are used to treat infections caused by bacteria, virus, protozoa, parasites, etc. They are also used in management of various malignant diseases (cancer). The major problem associated with use of chemotherapeutic agents is resistance to chemotherapeutic agents.

Mechanisms underlying resistance to chemotherapeutic agents include inactivation/modification of antibiotic (beta-lactams, chloramphenicol), insensitive target site (beta-lactams, glycopeptides, macrolides, tetracyclines), decreased drug accumulation in the form of enhanced efflux (tetracyclines, chloroquine, macrolides, anticancer drugs), by-pass of antibiotic sensitive step (methicillin, sulphonamides) etc. The common mechanisms underlying drug resistance is to restrict concentration of drug at the site of action usually intracellular. This can be in the from of restricting the entry of the drug into the cell by various mechanisms including altered cell wall permeability. It can also be in the form of removing the drug from site of action e.g. intracellular so that therapeutic concentration are not achieved.

This is largely done by throwing out intracellular drug at a rate faster than usual so that balance of drug concentration is disturbed resulting into lower intracellular concentration. This is done through the mechanism which is known as efflux pump. The other mechanism of decreasing therapeutic concentration of a drug include metabolism/alteration of drug to inactive compound e.g. secretion of enzymes like penicillinase or B-lactamase which destroys penicillins or B-lactam antibiotics.

Attempts have been made and efforts are on to counteract this acquired drug resistance. One way is to improve chemotherapeutic agents e.g. penicillin's resistant penicillin like Cloxacillin, dicloxacillin, methicillin, flucloxacillin etc. or beta-lactamase resistance antibiotics like temocillin in a group o penicillin or advanced cephalosporins, monobactams etc.

The other way is to find out drugs to overcome resistance for use along with chemotherapeutic agents e.g. beta-lactamase inhibitors like clavulanic acid, salbactam, tazobactam to be used along with antibiotics like amipicillin, amoxycillin, ticarcillin etc.

However, this approach has been successful in situations wherein beta-lactamase enzyme has been the problem.

The present situation is faced with new mechanism of resistance, like enhancement of efflux responsible for multi-drug resistance. This was initially seen with tetracycline antibiotics, but now is extended to many antibiotics like ciprofloxacin, norfloxacin, chloroquine and also has extended to many anticancer therapies (shown in the list below) which is now the major mechanism of resistance.

To overcome this efflux, attempts have been made by use of compounds called chemosensitizers. For example, calcium channel blocker, verpamil has been used to reverse chloroquine resistance in Plasmodium falciparum.

Likewise, modified tetracyclines has been used as chemosensitizers to overcome efflux mediated drug resistance.

In case of anticancer therapies, several compounds have been identified that enhanced drug's accumulation inside the cell like verapamil, amiodarone, steroids, cyclosporins, phenothiazines and other compounds as shown in the list below.

The drugs to be used along with chemotherapeutic agents (Table 1) for restoring sensitivity to chemotherapeutic agents are broadly known as chemosensitizers (Table 2). Many drugs used for various therapeutic effect are found to be good for this purpose e.g. Verapamil, Reserpine, Cyclosporin.

Some of the chemotherapeutic agents like rifampicin can work as a chemotherapeutic agent as well as chemosensitizer.

Table 1:

Chemotherapeutic Agents		
Doxorubicin	Mitoxantrone	Ciprofloxacin
Daunorubicin	Bleomycin	Norfloxacin
Vincristine	Rifampicin	Fluconazole
Vinblastine	Isoniazid	Itraconazole
Paclitaxel	Rifabutin	Ketoconazole
Carboplatin	Chloroquine	Clarithromycin
Etoposide	Tobramycin	Azithromycin
Taxotrene	Cloxacillin	Erythromycin
Topotecan	Dicloxacillin	Tetracycline
Adriamycin	Clavulanic acid	Carbenicillin
Cisplatin	Salbactam	Cefodizime
5-Fluorouracil	Tazobactam	Quinine

Table 2:

Chemosensitizers	
Carbonyl cyanide m-chlorophenylhydrazine	Trans-(E)- Flupentixol
Reserpine	Malagashanine
Verapamil	GF-120918
Cyclosporin A	HD-37
Omeprazole	L-Canavanine
Orthovandate	PSC-833
Trifluoperazine	Biricodar (VX-710)
5-methoxyhydnocarpin (5'-MHC)	GG 918
Valspodar	

However, the main problem associated with their use is higher therapeutic dose. It is not possible to administer known chemosensitizers in an amount necessary to obtain therapeutic levels required for chemosensitizing effect. Because of these they have not found wide spread applications. The search is going on to find out newer drugs for these purposes which can be used safely in therapeutic dose.

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SUMMARY OF THE INVENTION

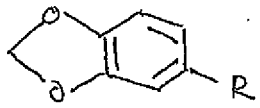
The present invention provides a chemosensitizer for therapeutic use, the dose of which as chemosensitizer is achievable. The chemosensitizer as per the present invention belongs to R-1-3 benzodiaxoles.

Piperine is a compound belonging to R-1-3 benozodiaxoles. Strains of M. tuberculosis growing in presence of 40 mcg/ml of rifampicin are inhibited at various concentrations of piperine, the concentration of which is not more than 5 mcg/ml.

Similarly in case of chloroquine-resistant P. falciparum strains, amount of piperine is dependent on level of resistance and amount of chloroquine.

DESCRIPTION OF THE INVENTION

According to the present invention it is observed that R-1-3 Benzodioxole compounds with structural formula



are chemosensitizers.

The amount of chemosensitizer required is dependent on the compound, chemotherapeutic agent and level of resistance.

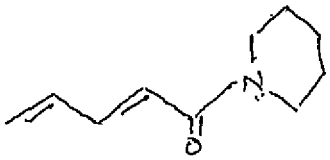
The following examples provide proof of such compounds working as chemosensitizer.

EXAMPLE 1

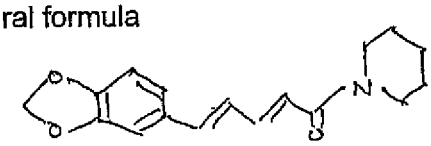
Rifampicin is a chemotherapeutic agent useful in the management of tuberculosis. Resistance to rifampicin is a major health problem.

A strain of mycobacterium is considered resistant if it grows in presence of rifampicin 40 mcg/ml.

Piperine is a compound belonging to a class of compound which can be grouped as R-1-3 benzodiaxole, where in, R is



Piperine with structural formula



is found to reverse resistance to rifampicin. The amount of piperine required for this action does not have any effect on Mycobacterium tuberculosis.

The table below gives results of such study against nine different such strains. It is clear that all strains growing in vitro in presence of 40 mcg/ml of rifampicin are inhibited at various concentration of piperine. The concentration of piperine required for this purpose is not more than 5 mcg/ml.

RIFA resistant strain	**Control RIFA 40 mcg/ml**	**RIFA 8 mcg/ml Piperine mcg/ml**			**RIFA 40 mcg/ml Piperine mcg/ml**		
		1	**2**	**5**	**1**	**2**	**5**
1	+++	++	NG	NG	NG	NG	NG
2	+++	++	NG	NG	NG	NG	NG
3	+++	++	+	NG	+	NG	NG
4	+++	++	NG	NG	±	NG	NG
5	+++	+	NG	NG	NG	NG	NG
6	+++	NG	NG	NG	NG	NG	NG
7	+++	++	+	NG	+	NG	NG
8	+++	++	NG	NG	NG	NG	NG
9	+++	+	±	NG	±	NG	NG

EXAMPLE 2:

Chloroquine is used in the management of malaria. One of the organism, Plasmodium falciparum has acquired resistance to chloroquine. This is the major cause of morbidity and mortality caused by malaria.

The table below shows how piperine at different concentrations overcomes P. falciparum resistance to chloroquine. The figures in each cell shows % inhibition of P. falciparum. It clearly shows that % inhibition for a resistant strain of P.falciparum can be improved by addition of piperine. Inhibition can be increased by increasing the dose of either compound.

Concentration of Chloroquine (p.mols)		**CHLOROQUINE + PIPERINE**					
	CQ	**20 mg**	**60 mg**	**100mg**	**200 mg**	**600 mg**	**1000mg**
1	09.6	6.3	79.0	100	100	100	100
2	27.7	17.7	90.0	100	100	100	100
4	33.7	22.8	93.8	100	100	100	100
8	57.8	65.8	100	100	100	100	100
16	83.1	100	100	100	100	100	100
32	95.2	100	100	100	100	100	100
64	100	100	100	100	100	100	100

The amount of piperine required is dependent on level of resistance and amount of chemotherapeutic agent.

Piperine was evaluated for its bioavailability and toxicity in animals.

The results of the pharmacokinetic study in rat (Figure 1) shows that compound is absorbed from intestinal tract when given orally. It also indicates that compound has a long half-life.

The toxicological study also reveals that piperine is non toxic.

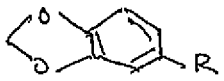
Pharmacokinetic study in humans (table below and Figure 2) also reveal that piperine is absorbed from gastrointestinal tract, and adequate therapeutic concentration can be achieved.

Dose (mg)	Time in hours										
	0	1	2	3	4	6	8	12	24	30	36
100	0	0.168 ± 0.099	1.403 ± 0.278	1.95 ± 0.434	1.691 ± 0.314	1.633 ± 0.227	1.534 ± 0.225	1.457 ± 0.342	0.688 ± 0.186	0.428 ± 0.083	-
250	0	0.684 ± 0.439	2.322 ± 0.65	3.754 ± 1.108	3.768 ± 0.873	3.797 ± 0.919	3.337 ± 0.558	2.935 ± 0.526	1.873 ± 0.348	1.40 ± 0.281	0.913 ± 0.189
500	0	1.292 ± 0.48	4.243 ± 1.027	7.406 ± 1.284	9.481 ± 1.635	7.717 ± 1.024	7.494 ± 0.982	6.813 ± 0.99	5.393 ± 0.988	4.381 ± 0.707	3.149 ± 0.72

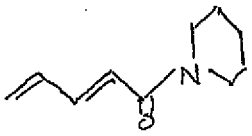
Thus, according to the present invention is provided R-1-3 benzodioxole compounds useful as chemosensitizers. These compounds can be useful in therapy as it is possible to achieve therapeutic levels in plasma.

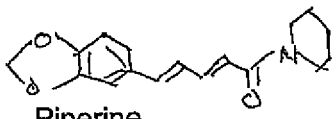
I claim:

1. R-1-3 Benzodioxoles as a chemosensitizer with a structural formula



2. A chemosensitizer as claimed in claim 1 can be piperine wherein R is





Piperine

3. A chemosensitizer as claimed in claim 1 to 2 is useful in overcoming resistance of chemotherapeutic agents.

4. Chemotherapeutic agent as claimed in claim 3 can be rifampicin.

5. Chemotherapeutic agents as claimed in claim 3 can be antimetabolites.

6. Antimetabolites as claimed in claim 5 can be selected from group comprising Doxorubicin, Dounorubicin, Vincristine, Vinblastine, Paclitaxel, Carboplatin, Etoposide, Taxotrene, Topotecan, Adriamycin, Cisplatin, 5-Fluorouracil, Mitoxantrone, Bleomycin and the like.

7. Chemotherapeutic agent as claimed in claim 3 can be chloroquine.

FIGURE 1: PHARMACOKINETC STUDY IN RAT

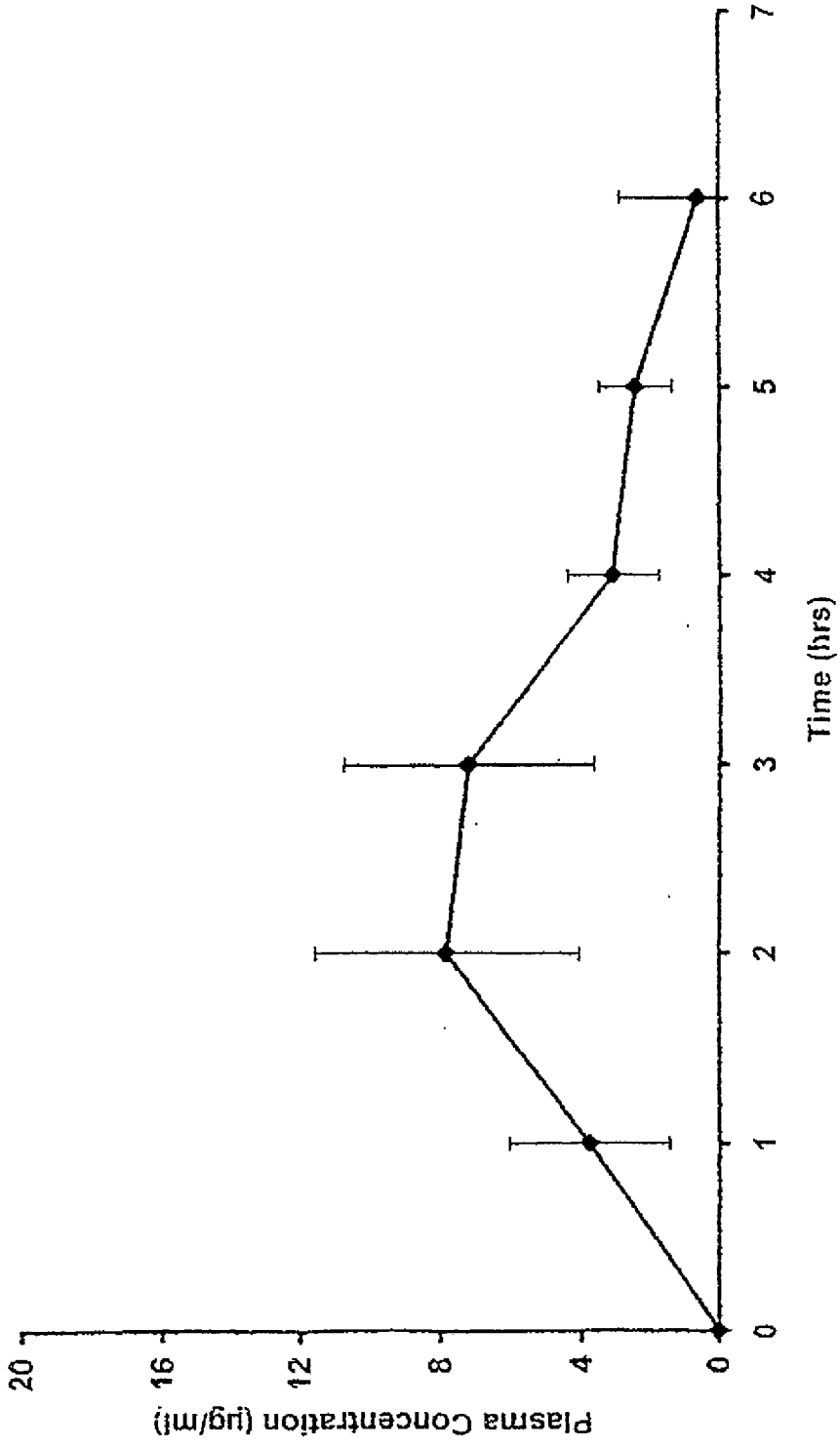


FIGURE 2: PLASMA CONCENTRATION -TIME PROFILE IN HUMAN VOLUNTEERS AFTER ORAL ADMINISTRATION